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Effects of formoterol or salmeterol on impulse oscillometry in patients with persistent asthma



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Background: Effects of small-particle long-acting β -agonists on the small airways have been poorly documented.

Objective: We used impulse oscillometry (IOS) to compare single and repeated dosing effects of small- and large-particle long-acting β -agonists.

Methods: After a 1- to 2-week run-in period, patients received either 12 μ g of small-particle hydrofluoroalkane 134a–formoterol solution or 50 μ g of large-particle salmeterol dry powder twice daily plus inhaled corticosteroid for 1 to 2 weeks with a 1- to 2-week washout period in between. Measurements were made over 60 minutes after the first and last doses.

Results: Sixteen patients completed the study as follows: mean age, 43 years; FEV₁, 80%; forced midexpiratory flow between 25% and 75% of forced vital capacity (FEF₂₅₋₇₅), 48%; total airway resistance at 5 Hz, 177%; peripheral airway resistance as the difference between 5 and 20 Hz, 0.18 kPa·L⁻¹·s; Asthma Control Questionnaire score, 0.76; and inhaled corticosteroid dosage, 550 μ g/d. There were significantly greater improvements with formoterol versus salmeterol in all IOS outcomes and FEF₂₅₋₇₅, but not FEV₁, at 5 minutes after the first dose, which were not sustained over 60 minutes. After the last dose, all IOS outcomes, but not FEV₁ or FEF₂₅₋₇₅, were significantly better with formoterol over the entire 60 minutes: mean difference at 60 minutes between formoterol and salmeterol in total airway resistance at 5 Hz, 7.50% (95% CI, 1.56% to 13.43%, $P = .02$); central airway resistance at 20 Hz, 5.37% (95% CI, 0.13% to 10.62%, $P = .045$); peripheral airway resistance as the difference between 5 and 20 Hz, 12.76% (95%

CI, 1.28% to 24.24%, $P = .03$); reactance area under the curve, 19.46% (95% CI, 7.56% to 31.36%, $P = .003$); reactance at 5 Hz, 11.19% (95% CI, 4.62% to 17.76%, $P = .002$); and resonant frequency, 9.34% (95% CI, 3.21% to 15.47%, $P = .005$). Peak expiratory flow significantly improved to a similar degree with both drugs.

Conclusion: Significant improvements in IOS outcomes but not spirometry results occurred after chronic dosing with formoterol compared with salmeterol. This might reflect better deposition to the entire lung, including the small airways. (*J Allergy Clin Immunol* 2016;137:727-33.)

Key words: Asthma, small airways, spirometry, impulse oscillometry, long-acting β -agonist, formoterol, salmeterol

Inhaled corticosteroid (ICS) with long-acting β -agonist (LABA) combination inhalers are now well established in current management guidelines as the preferred form of controller therapy in patients with persistent asthma. There is now increasing evidence to support a distinct asthma phenotype characterized by the presence of a disproportionate degree of persistent small-airways dysfunction.¹ It is possible to assess small-airways dysfunction in the so-called “silent zone” by using impulse oscillometry (IOS) to measure peripheral airway resistance as the difference between 5 and 20 Hz (R5-20) or peripheral airway capacitance as either the reactance area under the curve (AX) or reactance at 5 Hz (X5), as well as resonant frequency (RF).^{2,3}

Spirometry can be used to derive volume-dependent airways closure as the forced expiratory flow between 25% and 75% of forced vital capacity (FEF₂₅₋₇₅). IOS is an effort-independent test performed during normal tidal breathing to measure the frequency dependence of sound waves emanating from the bronchial tree and is considered more physiologic than spirometry, which tends to exaggerate small-airways closure associated with forced expiration.¹ Moreover, IOS is much easier and quicker to perform than spirometry, making it well suited to routine measurement in a busy clinic setting. Patients with the small-airways asthma phenotype who exhibit well-preserved FEV₁ in conjunction with abnormal R5-20 or FEF₂₅₋₇₅ values, which in turn confers an increased risk of poorer long-term asthma control.⁴ In a prospective study one IOS parameter (AX) showed sustained improvement over a prolonged period of follow-up in asthmatic children receiving controller therapy compared with spirometry.⁵

The advent of a small-particle hydrofluoroalkane 134a (HFA)–beclomethasone/formoterol combination solution metered-dose inhaler (Chiesi, Cheadle, United Kingdom) has provided the opportunity to target the entire lung in terms of both anti-inflammatory and smooth muscle responses in asthmatic patients associated with respective effects of ICS and LABA moieties. Although several clinical studies have attempted to differentiate between the effects of ICS formulations with different particle

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Abbreviations used

ACQ:	Asthma Control Questionnaire
AX:	Reactance area under the curve
FEF ₂₅₋₇₅ :	Forced midexpiratory flow between 25% and 75% of forced vital capacity
FENO:	Fraction of exhaled nitric oxide
HFA:	Hydrofluoroalkane 134a
ICS:	Inhaled corticosteroid
IOS:	Impulse oscillometry
LABA:	Long-acting β -agonist
PEF:	Peak expiratory flow
R5:	Total airway resistance at 5 Hz
R5-20:	Peripheral airway resistance as the difference between 5 and 20 Hz
R20:	Central airway resistance at 20 Hz
RF:	Resonant frequency
X5:	Reactance at 5 Hz

sizes,⁶⁻¹¹ relatively little attention has been given to the effects of LABA formulations on the small airways.¹

The aim of the present proof-of-concept study was to assess the effect of particle size on bronchodilator response with IOS using 2 different LABAs currently licensed for add-on to ICSs in the United Kingdom, namely the 12- μ g small-particle HFA-formoterol solution metered-dose inhaler (Chiesi) and 50- μ g large-particle salmeterol dry powder inhaler (GlaxoSmithKline, Uxbridge, United Kingdom), which were chosen to represent 2 extremes of mass median aerodynamic diameter at 0.80 and 3.64 μ m, respectively.^{12,13} It is generally considered that particles less than 2 μ m in diameter are able to optimally penetrate the small airways,¹ which is the reason for choosing the 2 formulations at different ends of the particle size spectrum. We elected to use what are normally considered therapeutically equivalent doses of formoterol (12- μ g nominal ex valve dose = 10.1- μ g delivered ex actuator dose) and salmeterol (50- μ g nominal ex valve dose = 47- μ g delivered dose).¹⁴

Specifically, we wanted to dissect out the independent effects of the different LABAs to obviate any potential confounding effect of different ICS moieties in their respective combination inhalers. Hence we decided to provide each LABA inhaler in addition to the same reference ICS therapy (as large-particle beclomethasone), with evaluation of airway responses after single and repeated dosing by using IOS and spirometry. Another reason for choosing the respective LABAs (rather than comparing small- and large-particle formoterol) is that the results from this study would presage a subsequent chronic dosing comparison of combination inhalers, namely 200/12 μ g beclomethasone/formoterol solution (Chiesi) versus 250/50 μ g fluticasone/salmeterol dry powder (GlaxoSmithKline).

METHODS

Study participants

Inclusion criteria were male or female volunteers aged at least 16 years with a diagnosis of persistent asthma, total airway resistance at 5 Hz (R5) of greater than 150% of predicted value, R5-R20 value of 0.05 $\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$ despite receiving ICS or ICS/LABA treatment, and FEV₁ of greater than 60%. Exclusion criteria were other significant respiratory diseases, an asthma exacerbation or respiratory tract infection requiring systemic steroids and/or antibiotics within 3 months of study commencement, and smoking within 1 year or a greater than 10 pack year history.

Study design

We carried out a single-center, randomized, open-label, crossover study (Fig 1). Patients were randomized to either 50 μ g of dry powder inhaler salmeterol (GlaxoSmithKline) twice daily or 12 μ g of HFA-formoterol solution (Chiesi) twice daily. The primary outcome was percentage change in R5-R20 from baseline. Secondary outcomes included percentage change from baseline in the remaining IOS variables (R5, central airway resistance at 20 Hz [R20], X5, RF, and AX), spirometry (FEV₁ and FEF₂₅₋₇₅), domiciliary peak expiratory flow (PEF), Asthma Control Questionnaire (ACQ) score, and fraction of exhaled nitric oxide (FENO).

At the screening visit, participants were converted to a reference HFA-beclomethasone dipropionate inhaler (Chiesi) with a mass median aerodynamic diameter of 2.9 μ m¹⁵ at an equivalent beclomethasone dipropionate dose, and any concomitant LABA was also stopped. After a 1- to 2-week run-in period, at visit 1, baseline measurements for IOS, spirometry, ACQ score, and FENO value were recorded. The first dose of the study inhaler was then administered in the department. IOS and spirometry were repeated at 5, 15, 30, 45, and 60 minutes after the first dose. After 1 to 2 weeks on the study inhaler, participants returned to the department for visit 2, with the penultimate dose of study inhaler being taken 12 hours before the study visit. Baseline measurements for the chronic dosing visit were recorded, and the last dose of the study inhaler was then administered. IOS and spirometry outcomes were recorded over 60 minutes as per after the first dose. Participants subsequently entered a wash-out period of 1 to 2 weeks, after which the same process was repeated with the other study inhaler after crossover at visits 3 and 4.

Measurements

IOS (Masterscreen IOS, Höchberg, Germany) was performed in triplicate, according to the manufacturer's guidelines. Spirometry was performed with a SuperSpiro (Micro Medical, Chatham, Kent, United Kingdom), and FENO values were measured with a NIOX analyzer (NIOX Nitric Oxide Monitoring System; Aerocrine AB, Solna, Sweden), according to American Thoracic Society guidelines.^{16,17} Asthma control was assessed by using the 6-item ACQ.^{18,19}

Ethics

The East of Scotland Research Ethics Service granted ethical approval (reference 13/ES/0050), and all patients provided written informed consent.

Statistical analyses

Sample size estimates were based on previous IOS data,²⁰ such that 16 patients would be required to complete per protocol to detect a 20% difference in the primary outcome of R5-20 achieving 80% power with an α error of .05 (2-tailed). We compared baselines according to treatment before the first and last doses of formoterol versus salmeterol. The respective baselines were then used to calculate the percentage changes for IOS and spirometry after single and chronic dosing. Repeated-measures ANOVA was applied to assess any treatment-time interaction over the 60-minute profile after single or repeated dosing. Hence the absence of a significant interaction indicates that any detectable differences between treatments are consistent over the 60 minutes, in turn resulting in a 95% CI for the overall treatment difference that excludes zero. Data for domiciliary peak flow were calculated as the average values from the last 3 days of the run-in, washout, and each repeated treatment period and were then compared by using paired Student *t* tests. SPSS version 21 (SPSS, Chicago, Ill) was used for all analyses.

RESULTS

Participant flow through the study is shown in Fig 2. Sixteen patients completed per protocol: mean age, 43 years; FEV₁, 80%; R5, 177%; R5-20, 0.18 $\text{kPa} \cdot \text{L}^{-1} \cdot \text{s}$; and ACQ score, 0.76 (Table I).

Baseline values before single or repeated dosing were not significantly different when comparing formoterol versus salmeterol (Table II). There were no differences when comparing

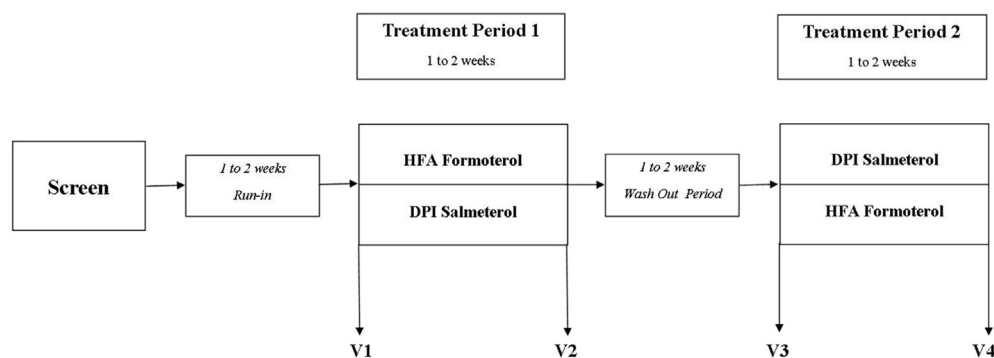


FIG 1. After a 1- to 2-week run-in period, patients received either 12 µg of small-particle HFA-formoterol or 50 µg of large-particle salmeterol dry powder 1 puff twice daily for 1 to 2 weeks each, with a 1- to 2-week washout period in between. Visits 1 and 3 were to assess the first dose, and visits 2 and 4 were to assess the last dose. At each visit, measurements were made at baseline and then over 60 minutes after inhalation. *DPI*, Dry powder inhaler.

CONSORT Flow Diagram

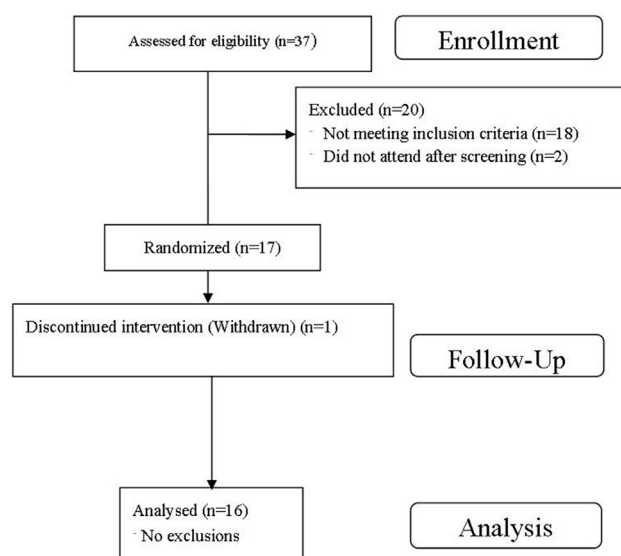


FIG 2. CONSORT diagram to show flow of participants through the study.

baseline values between visits 1 and 3 after the run-in and washout periods, respectively.

After single dosing, the overall treatment response (ANOVA over 60 minutes) comparing formulations was not significantly different with IOS or spirometry, as indicated by 95% CIs for the difference that included zero for the percentage change from respective baseline (see Table E1 in this article's Online Repository at www.jacionline.org). Moreover, there was a significant treatment-time interaction for all IOS outcomes, indicating that any differences between formulations were not consistent over the 60-minute time period (Fig 3). A significantly greater response was seen with formoterol versus salmeterol at 5 minutes for all IOS outcomes and for FEF₂₅₋₇₅ but not FEV₁, which is in keeping with a faster speed of onset (Table III).

After repeated dosing, there were significant overall improvements (ANOVA over 60 minutes) conferred by formoterol over salmeterol for all IOS outcomes, as indicated by 95% CIs for the difference that excluded zero for the percentage change from

TABLE I. Demographics at screening

Age (y)	43 (4.2)
Male/female sex	9/7
BDP equivalent dose	550 (74)
LABA (%)	50
ACQ-6 score	0.76 (0.17)
FENO (ppb)	27 (3.0)
FEV ₁ (% predicted)	80 (2.5)
FVC (% predicted)	94 (3.1)
FEF ₂₅₋₇₅ (% predicted)	48 (4.5)
FEV ₁ /FVC ratio	0.72 (0.02)
R5 (kPa·L ⁻¹ ·s)	0.56 (0.04)
R5 (% predicted)	177 (12)
R20 (kPa·L ⁻¹ ·s)	0.38 (0.02)
R5-R20 (kPa·L ⁻¹ ·s)	0.18 (0.03)
X5 (kPa·L ⁻¹ ·s)	-0.24 (0.03)
RF (Hz)	21.5 (1.2)
AX (kPa·L ⁻¹)	1.92 (0.35)

All values are shown as means and SEMs.

BDP, Beclomethasone dipropionate; FVC, forced vital capacity.

respective baseline (Table IV). The treatment-time interaction was nonsignificant for all IOS outcomes, indicating that such differences were consistent over the entire 60-minute period (Fig 4).

No significant differences between formoterol and salmeterol were seen in spirometry outcomes (FEV₁ or FEF₂₅₋₇₅) after repeated dosing (Table IV).

Mean domiciliary peak flow measurements showed significant but similar improvements with both formoterol (from 442 to 474 L/min, $P = .002$) and salmeterol (from 450 to 474 L/min, $P < .001$) after repeated dosing versus baseline. Mean ACQ-6 scores were not significantly different after repeated dosing when comparing values between formoterol (0.48) and salmeterol (0.52). FENO values were not significantly altered after formoterol or salmeterol, with levels of 22.5 and 23.6 ppb, respectively.

DISCUSSION

The results of the present study showed significantly greater improvements in all IOS outcomes conferred by small-particle formoterol in terms of total (R5), central (R20), and peripheral airway resistance (R5-20) and AX and X5, as well as RF. These

TABLE II. Baselines according to treatment for IOS and spirometry

	Acute baseline			Chronic baseline		
	Salmeterol	Formoterol	P value	Salmeterol	Formoterol	P value
FEV ₁ (L)	2.61 (2.27 to 2.94)	2.62 (2.30 to 2.94)	.84	2.72 (2.38 to 3.06)	2.69 (2.33 to 3.06)	.47
FEF ₂₅₋₇₅ (L·s ⁻¹)	1.85 (1.54 to 2.16)	1.76 (1.42 to 2.09)	.17	1.92 (1.56 to 2.28)	1.97 (1.57 to 2.36)	.53
R5 (kPa·L ⁻¹ ·s)	0.60 (0.51 to 0.68)	0.58 (0.51 to 0.65)	.42	0.50 (0.44 to 0.56)	0.52 (0.46 to 0.58)	.40
R20 (kPa·L ⁻¹ ·s)	0.39 (0.36 to 0.43)	0.39 (0.35 to 0.42)	.76	0.36 (0.33 to 0.39)	0.37 (0.34 to 0.41)	.38
R5-R20 (kPa·L ⁻¹ ·s)	0.20 (0.14 to 0.27)	0.19 (0.12 to 0.25)	.46	0.14 (0.09 to 0.19)	0.15 (0.11 to 0.18)	.68
X5 (kPa·L ⁻¹ ·s)	-0.25 (-0.31 to 0.19)	-0.24 (-0.30 to 0.18)	.76	-0.19 (-0.23 to -0.16)	-0.20 (-0.24 to -0.15)	.68
RF (Hz)	24.4 (21.1 to 27.7)	23.3 (20.4 to 26.2)	.30	20.60 (17.67 to 23.53)	20.24 (17.53 to 22.95)	.60
AX (kPa·L ⁻¹)	2.35 (1.52 to 3.18)	2.13 (1.38 to 2.88)	.54	1.45 (0.98 to 1.92)	1.48 (0.96 to 2.00)	.83

P values refer to the comparison between salmeterol and formoterol for baseline values before the first (acute) or last (chronic) dose. Values are shown as means (95% CIs).

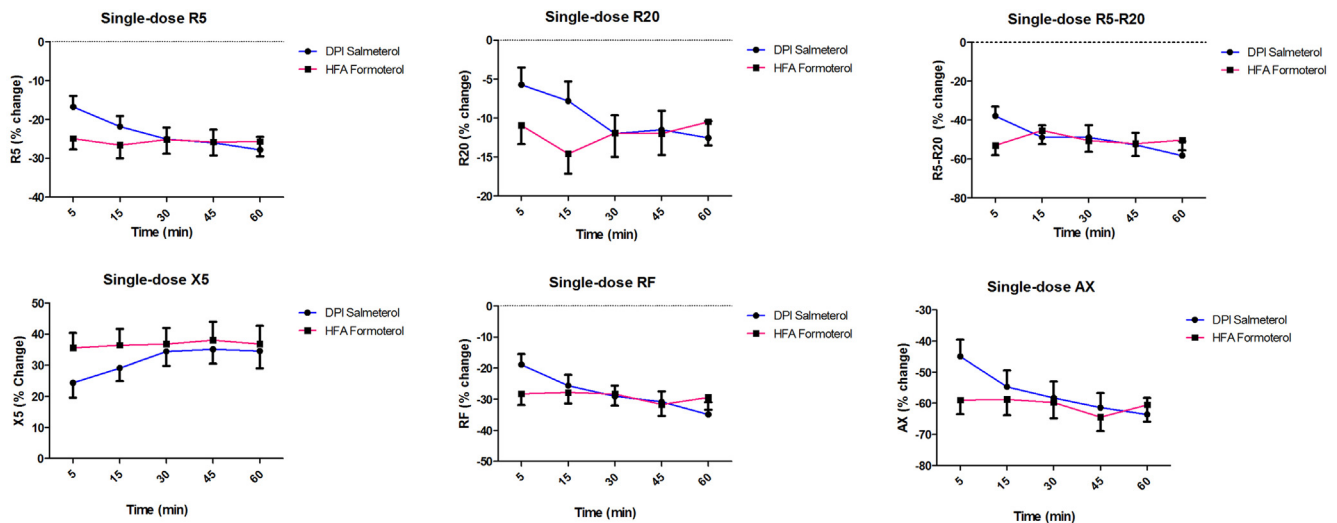


FIG 3. Effects on IOS outcomes with either small-particle formoterol or large-particle salmeterol over a 60-minute period after inhaling the first dose shown as the percentage change from baseline. Data are depicted as means and SEMs. For all IOS outcomes, there were significant differences at 5 minutes, which were not sustained over the entire 60-minute period.

TABLE III. Percentage change from baseline at 5 minutes after the first dose

	Salmeterol (% change)	Formoterol (% change)	Difference (%)	P value
FEV ₁	3.40	6.43	3.04 (-1.39 to 7.46)	.16
FEF ₂₅₋₇₅	4.74	13.37	8.63 (0.85 to 16.41)	.03
R5	16.77	24.93	8.16 (3.21 to 13.11)	.003
R20	5.71	10.95	5.23 (0.03 to 10.43)	.049
R5-R20	37.87	53.05	15.18 (5.76 to 24.59)	.004
X5	24.37	35.62	11.25 (1.79 to 20.72)	.02
RF	18.90	28.21	9.31 (3.72 to 14.90)	.003
AX	44.93	58.92	13.99 (4.24 to 23.75)	.008

Mean values are shown for each drug, as well as the difference (as 95% CIs).

TABLE IV. Percentage change from baseline over 60 minutes after the last dose

	Salmeterol (% change)	Formoterol (% change)	Difference (%)	P value
FEV ₁	3.15	5.81	2.65 (-0.66 to 5.96)	.11
FEF ₂₅₋₇₅	9.93	13.5	3.57 (-4.99 to 12.14)	.39
R5	6.11	13.60	7.50 (1.56 to 13.43)	.02
R20	1.45	6.82	5.37 (0.13 to 10.62)	.045
R5-R20	18.36	31.12	12.76 (1.28 to 24.24)	.03
X5	9.29	20.48	11.19 (4.62 to 17.76)	.002
RF	8.70	18.04	9.34 (3.21 to 15.47)	.005
AX	18.81	38.27	19.46 (7.56 to 31.36)	.003

Mean values are shown for each drug, as well as the difference (as 95% CIs).

changes in IOS outcomes occurred after repeated dosing with 12 µg of twice-daily formoterol compared with 50 µg of twice-daily salmeterol when used as add-on to ICSs in controlled patients with mild-to-moderate persistent asthma. Moreover, the differences between formulations for all of the IOS outcomes were sustained over the entire 60-minute period after inhaling the last dose. The greater reduction in central and peripheral resistance might reflect enhanced distribution of formoterol throughout the lung, including the small airways, which is in

keeping with its finer particle size. No such differences were seen for FEV₁, FEF₂₅₋₇₅, or domiciliary PEF after repeated dosing.

The apparent disconnect in IOS outcomes between single and chronic dosing might be explained by the cumulative effects of extrafine particles on the smaller airways on repeated exposure, resulting in subtle changes in airway geometry. In other words, it is possible that equilibration was achieved at steady state for regional bronchodilator effects, which improved the overall signal/noise ratio seen with IOS after repeated but not single dosing.

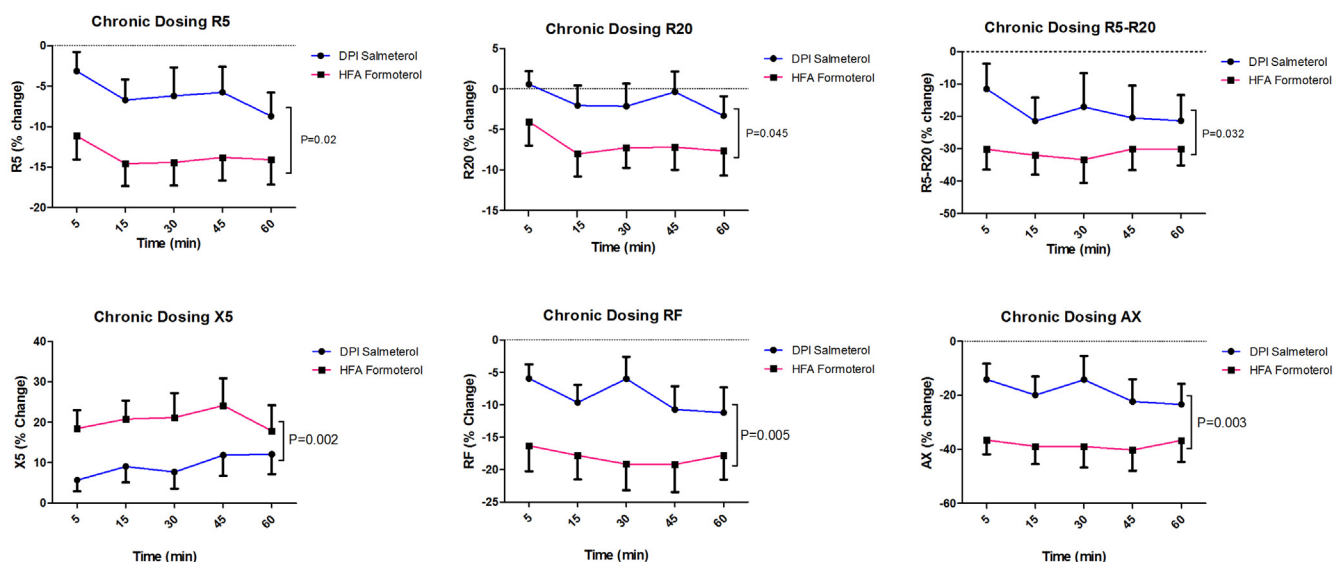


FIG 4. Effects on IOS outcomes with either small-particle formoterol or large-particle salmeterol over a 60-minute period after inhaling the last dose shown as percentage change from baseline. Data are depicted as means and SEMs. For all IOS outcomes, there were significant differences between treatments, which were sustained over the 60-minute period.

A limitation of our study is that formoterol and salmeterol have different pharmacologic properties. If the faster speed of onset with formoterol was the explanation for the greater improvement in IOS outcomes after the last dose, then one would expect to see the same difference between LABAs maintained over the entire 60-minute profile after both single and repeated dosing, which was not observed in the present study. The other pharmacologic possibility to explain the observed difference in IOS outcomes after repeated dosing could be the greater degree of β_2 -receptor intrinsic agonist activity when comparing formoterol (full agonist) and salmeterol (partial agonist). However, one would predict that the higher agonist activity of formoterol would produce more β_2 -receptor downregulation and associated subsensitivity of response than salmeterol after the last dose, whereas the opposite was seen.

In a previous comparison of single doses of large-particle metered-dose inhaler formulations of 24 μg of formoterol or 50 μg of salmeterol administered through a large-volume spacer in patients with moderately severe asthma, there was a significantly greater response on FEV_1 with formoterol at 10 minutes but not at any other time points in the first 60 minutes after inhalation.²¹ In the same study specific airway conductance showed sustained improvements with formoterol over the entire 60 minutes, with there being no difference between 4 and 12 hours after inhalation, although no chronic dosing assessment was performed. This confirms the greater sensitivity of measuring airway resistance (its reciprocal as Gaw) over dynamic lung volumes (as FEV_1), which was also seen in our study when comparing R5 and FEV_1 , although we used formoterol without a spacer. The use of a large-volume spacer would result in an increase in overall respirable dose and an associated reduction in particle size. Hence 24 μg of formoterol would normally be considered therapeutically equivalent to 100 μg rather than 50 μg of salmeterol, which might explain the observed superiority in the study by Van Noord et al.²¹ The fast onset with formoterol forms the basis of its licensed indication for use as both maintenance and

reliever therapy during chronic dosing when using a single ICS/LABA combination inhaler, such as a beclomethasone/formoterol pressurized metered-dose inhaler.²²

We performed our measurements at steady state to correspond with the end of the usual 12-hour dosing interval, reflecting a twice-daily regimen with LABAs. This period at trough represents a time in the day when the patient might be at their most vulnerable to potential bronchoconstrictor stimuli before taking the next dose. At steady state with repeated dosing, the airway caliber will be slightly higher because of some residual β_2 -receptor occupancy at trough, as was shown when comparing the respective baseline values before the first and last doses of each LABA.

We defined bronchodilator response as the percentage change from respective baseline values after the first and last dose because we believed this best reflected the relative changes in airway caliber. Thus there will inevitably be an apparent degree of blunting of the magnitude of the percentage response from the higher baseline value when comparing effects after repeated versus single dosing. Pointedly, we found no significant differences between the respective baseline values for salmeterol versus formoterol before the last dose. In turn, this allowed us to make valid head-to-head comparisons between the 2 formulations after repeated dosing. It is also worth mentioning that we observed no differences when comparing baseline values between visits 1 and 3 after the run-in and washout periods, respectively, confirming that there were no carryover effects between the randomized treatment arms. This is to be expected because the duration of washout of at least 1 week (ie, 168 hours) exceeded a period of 5 half-lives for either LABA (ie, 60 hours).

Our data showed that after the last dose, the 95% CI for the overall mean difference (ie, over 60 minutes) excluded zero for all IOS outcomes, which is in keeping with significantly sustained improvements in both total and peripheral airways resistance and reactance. The density of β_2 -receptors is higher in the distal than proximal airways,²³ perhaps also reflecting the greater overall relative surface area in the small airways. This needs to be

considered against the amount by which airway smooth muscle reduces proportionately from the proximal to distal airways.

We appreciate that the observed differences in IOS outcomes might not translate into improvements in symptom control. It has been shown that R5-R20 and AX values, but not FEV₁, correlate to health status, dyspnea, and disease control.²⁴ In another study comparing asthmatic patients with and without symptoms who underwent a methacholine challenge, the symptomatic group exhibited a significantly greater change in R5-20 and AX values after challenge.²⁵

Our patients had stable mild-to-moderate persistent asthma, as reflected by the mean value of 0.76 for ACQ scores at initial screening, which is close to the 0.75 cutoff value for well-controlled disease.¹⁹ The normal mean FENO value of 27 ppb at screening is indicative of the suppressive effects of ICSs at a mean beclomethasone-equivalent dose of 550 $\mu\text{g}/\text{d}$.²⁶ Despite this, our patients had impairment of airway function in terms of FEV₁ (80% of predicted value), FEV₁/forced vital capacity ratio (0.72), and R5 (177% of predicted value), along with evidence of abnormal small-airways function on the basis of their R5-20 (0.18 $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$), AX (1.92 $\text{kPa}\cdot\text{L}^{-1}$), and FEF₂₅₋₇₅ (48% of predicted value) values. It has previously been found that peripheral airway dysfunction as R5-20 ($>0.07 \text{ kPa}\cdot\text{L}^{-1}\cdot\text{s}$) and FEF₂₅₋₇₅ ($<70\%$ of predicted value) is associated with poorer long-term asthma control in patients with a preserved FEV₁ of greater than 80% of predicted value.⁴

We did not initially screen patients for bronchodilator reversibility as an inclusion criterion because this is not something that is routinely done in our everyday clinical practice. It is conceivable that had we evaluated a group of patients with more severe disease, more impaired airway function, and greater reversibility, there might also have been a more pronounced difference in IOS outcomes. Another possibility to accentuate the signal might be to compare the formulations after methacholine bronchial challenge to assess whether the differences in IOS outcomes become more apparent in the presence of increased airway tone to better reflect what happens in the setting of an acute episode of bronchoconstriction in conjunction with distal air trapping.⁶ Another possibility would be to measure static lung volumes by using plethysmography (eg, residual volume/total lung capacity) or perhaps ventilation heterogeneity with nitrogen washout.¹

We found that IOS was more sensitive than spirometry to effects of particle size when comparing formulations after chronic dosing; indeed, we found no significant differences in FEV₁, FEF₂₅₋₇₅, or domiciliary PEF values. The apparent disconnect between IOS and spirometry outcomes might simply reflect our IOS-based selection criteria for inclusion as an R5 value of greater than 150% and an R5-20 value of greater than 0.05 $\text{kPa}\cdot\text{L}^{-1}\cdot\text{s}$. We have previously shown in terms of either bronchoconstriction or bronchodilation that the signal/noise ratio is generally superior for IOS compared with spirometry.²⁰ Our finding of no significant overall difference (ie, over 60 minutes) in IOS outcomes after single dosing is in keeping with those of Houghton et al,²⁷ who also showed no difference in IOS, plethysmographic, or spirometry outcomes over 90 minutes of recovery after methacholine challenge when comparing single doses of small-particle HFA-formoterol versus coarse-particle formoterol dry powder, although this was in unselected patients with mild-to-moderate persistent asthma. Like our single-dose data, they showed no further decrease in R5 values 15 minutes after single dosing.

We duly acknowledge that using separate ICS and LABA inhalers is not in keeping with current asthma management guidelines. The rationale here was to keep the ICS moiety as a reference while varying the LABA. We elected to use large-particle ICSs as the reference because it was considered that we might not see any further improvements in IOS outcomes with small-particle formoterol in addition to small-particle ICSs. In the present study one might argue that there was an unopposed effect of small-particle formoterol in the small airways in conjunction with large-particle ICSs. This being the case, one might predict a greater degree of tachyphylaxis of response because of the lack of ICSs in terms of reversing β_2 -receptor downregulation in the distal airways. In fact, we observed a greater IOS response after chronic dosing with formoterol than salmeterol, suggesting that this was unlikely to be the case.

In chronic dosing studies comparing small-particle beclomethasone-formoterol pressurized metered-dose inhaler versus large-particle fluticasone-salmeterol dry powder inhaler, there was a significant improvement in methacholine hyper-responsiveness and a significant reduction in closing volume with the former.^{28,29} However, such studies with combination inhalers cannot distinguish between the effects of ICS and LABA moieties *per se*.

In summary, the results of the present study have demonstrated significant differences in IOS outcomes, but not spirometry or PEF results, after repeated dosing with small-particle formoterol compared with large-particle salmeterol when given in addition to ICSs to patients with controlled mild-to-moderate persistent asthma. We believe this difference in IOS outcomes might be due in part to improved global deposition of small particles to the entire lung, including the smaller airways.

Clinical implications: Improved total and peripheral lung deposition with small-particle formoterol than large-particle salmeterol might translate into better outcomes measured by using IOS, which are not evident on spirometry.

REFERENCES

1. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. *Lancet Respir Med* 2014;2:497-506.
2. Landser FJ, Nagles J, Demedts M, Billiet L, van de Woestijne KP. A new method to determine frequency characteristics of the respiratory system. *J Appl Physiol* 1976;41:101-6.
3. Oostveen E, MacLeod D, Lorino H, Farré R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 2003;22:1026-41.
4. Manoharan A, Anderson WJ, Lipworth J, Ibrahim I, Lipworth BJ. Small airway dysfunction is associated with poorer asthma control. *Eur Respir J* 2014;44:1353-5.
5. Larsen GL, Morgan W, Heldt GP, Mauger DT, Boehmer SJ, Chinchilli VM, et al. Impulse oscillometry versus spirometry in a long-term study of controller therapy for pediatric asthma. *J Allergy Clin Immunol* 2009;123:861-7.e1.
6. Goldin JG, Tashkin DP, Kleerup EC, Greaser LE, Haywood UM, Sayre JW, et al. Comparative effects of hydrofluoroalkane and chlorofluorocarbon beclomethasone dipropionate inhalation on small airways: assessment with functional helical thin-section computed tomography. *J Allergy Clin Immunol* 1999;104(suppl):S258-67.
7. Yamaguchi M, Niimi A, Ueda T, Takemura M, Matsuoka H, Jinnai M, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. *Pulm Pharmacol Ther* 2009;22:326-32.
8. Nicolini G, Chetta A, Simonazzi A, Tzani P, Aiello M, Olivieri D. Both bronchial and alveolar exhaled nitric oxide are reduced with extrafine beclomethasone dipropionate in asthma. *Allergy Asthma Proc* 2010;31:85-90.
9. Price D, Martin RJ, Barnes N, Dorinsky P, Israel E, Roche N, et al. Prescribing practices and asthma control with hydrofluoroalkane-beclomethasone and fluticasone: a real-world observational study. *J Allergy Clin Immunol* 2010;126:511-8, e1-10.

10. Price D, Thomas M, Haughney J, Lewis RA, Burden A, von Ziegenweidt J, et al. Real-life comparison of beclometasone dipropionate as an extrafine- or larger-particle formulation for asthma. *Respir Med* 2013;107:987-1000.
11. Colice G, Martin RJ, Israel E, Roche N, Barnes N, Burden A, et al. Asthma outcomes and costs of therapy with extrafine beclomethasone and fluticasone. *J Allergy Clin Immunol* 2013;132:45-54.
12. Taki M, Ahmed S, Marriott C, Zeng XM, Martin GP. The 'stage-by-stage' deposition of drugs from commercial single-active and combination dry powder inhaler formulations. *Eur J Pharm Sci* 2011;43:225-35.
13. Haussermann S, Acerbi D, Brand P, Herpich C, Poli G, Sommerer K, et al. Lung deposition of formoterol HFA (Atimos/Forair) in healthy volunteers, asthmatic and COPD patients. *J Aerosol Med* 2007;20:331-41.
14. Palmqvist M, Persson G, Lazer L, Rosenberg J, Larsson P, Lotvall J. Inhaled dry-powder formoterol and salmeterol in asthmatic patients: onset of action, duration of effect and potency. *Eur Respir J* 1997;10:2484-9.
15. Ganderton D, Lewis D, Davies R, Meakin B, Brambilla G, Church T. Modulite: a means of designing the aerosols generated by pressurized metered dose inhalers. *Respir Med* 2002;96(suppl D):S3-8.
16. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912-30.
17. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602-15.
18. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005;99:553-8.
19. Juniper EF, Bousquet J, Abetz L, Bateman ED. GOAL Committee. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006;100:616-21.
20. Short PM, Williamson PA, Lipworth BJ. Sensitivity of impulse oscillometry and spirometry in beta-blocker induced bronchoconstriction and beta-agonist bronchodilatation in asthma. *Ann Allergy Asthma Immunol* 2012;109:412-5.
21. van Noord JA, Smeets JJ, Raaijmakers JA, Bommer AM, Maesen FP. Salmeterol versus formoterol in patients with moderately severe asthma: onset and duration of action. *Eur Respir J* 1996;9:1684-8.
22. Papi A, Corradi M, Pigeon-Francisco C, Baronio R, Siergiejko Z, Petruzzelli S, et al. Beclometasone-formoterol as maintenance and reliever treatment in patients with asthma: a double-blind, randomised controlled trial. *Lancet Respir Med* 2013;1:23-31.
23. Hamid QA, Mak JC, Sheppard MN, Corrin B, Venter JC, Barnes PJ. Localization of beta 2-adrenoceptor messenger RNA in human and rat lung using in situ hybridization: correlation with receptor autoradiography. *Eur J Pharmacol* 1991;206:133-8.
24. Takeda T, Oga T, Niimi A, Matsumoto H, Ito I, Yamaguchi M, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration* 2010;80:120-6.
25. Boudewijn IM, Telenga ED, van der Wiel E, van der Molen T, Schiphof L, Ten Hacken NH, et al. Less small airway dysfunction in asymptomatic bronchial hyper-responsiveness than in asthma. *Allergy* 2013;68:1419-26.
26. Anderson WJ, Short PM, Williamson PA, Lipworth BJ. Inhaled corticosteroid dose response using domiciliary exhaled nitric oxide in persistent asthma: the FENO-type trial. *Chest* 2012;142:1553-61.
27. Houghton CM, Woodcock AA, Singh D. A comparison of plethysmography, spirometry and oscillometry for assessing the pulmonary effects of inhaled ipratropium bromide in healthy subjects and patients with asthma. *Br J Clin Pharmacol* 2005;59:152-9.
28. Scichilone N, Battaglia S, Sorino C, Paglino G, Martino L, Paternò A, et al. Effects of extra-fine inhaled beclomethasone/formoterol on both large and small airways in asthma. *Allergy* 2010;65:897-902.
29. Corda L, Gardenghi GG, Modina D, Montemurro LT, Novali M, Tantucci C. Effects on small airway obstruction of long-term treatments with beclomethasone/formoterol hydrofluoroalkane (metered-dose inhaler) versus fluticasone/salmeterol (dry-powder inhaler) in asthma: a preliminary study. *Allergy Asthma Proc* 2011;32:29-34.

TABLE E1. Percentage change from baseline over 60 minutes after the first dose

	Salmeterol (% change)	Formoterol (% change)	Difference (%)	P value
FEV ₁	7.63	8.55	0.92 (−4.09 to 5.94)	.70
FEF ₂₅₋₇₅	15.23	31.24	16.01 (−2.65 to 34.68)	.09
R5	23.51	25.20	1.70 (−4.99 to 8.38)	.60
R20	9.92	11.99	2.10 (−3.12 to 7.26)	.41
R5-R20	49.32	50.28	0.97 (−12.16 to 14.10)	.88
X5	31.51	36.76	5.26 (−4.32 to 14.84)	.26
RF	27.83	29.08	1.25 (−5.77 to 8.27)	.71
AX	56.55	60.44	3.89 (−7.36 to 15.14)	.47

Mean values are shown for each drug, as well as the difference (as 95% CIs).